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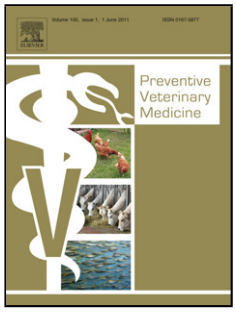

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A TARGETED INVESTIGATION TO DEMONSTRATE THE FREEDOM OF WEST TIMOR FROM HPAI H5N1

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Highlights

- This is a targeted survey that was undertaken in 2013 in 2 districts of West Timor, involving 300 village and commercial poultry (292 chickens and 8 Muscovy ducks) from 10 different villages between August and October 2013.
- The purpose of the study was to provide evidence that HPAI H5N1 was absent from West Timor
- Swabs of the cloaca and trachea of the sampled birds were tested using the Anigen® Rapid Test (Bionote).
- All samples were negative on testing (0%; 95%CI: 0.0 - 1.8%).
- From these results it was concluded with a high level of confidence (100%, 95%CI: 99.987, 100) that this population is not diseased, and these results, along with a lack of clinical evidence of disease, are adequate to conclude that West Timor is free from HPAI.

Abstract

In early 2004 highly pathogenic avian influenza (HPAI) H5N1 virus caused major outbreaks of disease in poultry in Indonesia. The disease was first reported in West Timor in eastern Indonesia in the same year, resulting in the death of

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ACCEPTED MANUSCRIPT

approximately one hundred chickens from both commercial and backyard farms; however no evidence of disease has subsequently been reported in West Timor since 2007.

A targeted survey was undertaken in 2013 in 2 districts of West Timor. Three hundred village and commercial poultry (292 chickens and 8 Muscovy ducks) from 10 villages and 5 live bird markets (LBMs) were sampled between August and October 2013. Swabs of the cloaca and trachea of the sampled birds were tested using the Anigen® Rapid Test (Bionote). All samples were negative on testing (0%; 95%CI: 0.0 - 1.2%). From these results it was concluded with a high level of confidence (100%, 95%CI: 99.988, 100) that this population is not infected, and these results, along with a lack of clinical evidence of disease, support the conclusion that West Timor was free from HPAI infection at the time of the survey.

Key words:
Highly pathogenic avian influenza H5N1; West Timor, Indonesia; Epidemiology. Poultry disease.

1. Introduction

The first outbreak of the disease in humans caused by H5N1 occurred in Hong Kong in 1997, with six of 18 infected individuals dying (Chan, 2002). Subsequently in 2004 the virus caused major outbreaks of disease in poultry in Indonesia (Smith *et al.*, 2006), Vietnam (Hien *et al.*, 2004), Thailand (Chotpitayasunondh *et al.*, 2005; Grose, 2004), Republic of Korea, Japan,

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Cambodia, and Lao People's Democratic Republic (WHO, 2005b). The outbreaks were associated with high levels of mortality, up to 100%, in poultry (WHO, 2014), as well as high case fatality rates (CFR) in humans (> 50%) (WHO, 2005a).

In Indonesia, HPAI H5N1 virus was first detected in domestic poultry in 2003 (Sedyaningsih *et al.*, 2007) and by the end of June 2006, the virus had been detected in 27 provinces (Indonesia Ministry of Agriculture, unpublished data, cited by Sedyaningsih *et al*. 2007). The virus was reported to be endemic in poultry in several provinces by 2009, with frequent outbreaks being reported on the islands of Java and Sumatera (Sumiarto and Arifin, 2008). In Indonesia HPAI not only resulted in the restriction of international trade of live birds and poultry meat products, but also affected tourism (Rushton *et al.*, 2005) and public health. Until 2015 there had been 199 confirmed human cases with a CFR of 83.9% (WHO, 2016). Most human cases in Indonesia (76%) have been associated with contact with poultry or poultry products (Sedyaningsih *et al.*, 2007). However recent studies have demonstrated person to person transmission, with clusters of epidemiologically linked H5N1 cases occurring among families (Kandun *et al.*, 2008).

In West Timor, HPAI (H5N1) caused the death of approximately one hundred chickens from both commercial and backyard farms between 2004 and 2006. It was first diagnosed in 2004 when five samples from chickens from two different farms tested positive on the haemagglutination inhibition (HI) test (unpublished data from Provincial Livestock Services, Nusa Tenggara Timur (NTT). Smith *et*

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al. (2006) suggested that the virus originated from West Indonesia (Java) through two separate introductions via the movement of poultry and/or poultry products.

Active surveillance for H5N1 in West Timor was first implemented in 2005, and was aimed primarily at the sector 4 poultry industry (household/backyard level) (Azhar *et al.*, 2010). Passive surveillance is also conducted through the reports of farmers to field veterinarians of dead chickens and the first diagnosis of the disease in 2004 resulted from farmers reporting deaths in their chickens to the Livestock Services (Personal communication, Drh. Cahyo Sunarno, Coordinator of the Participatory Disease Surveillance and Response (PDSR) program of NTT).

The results of serological surveys conducted by the Provincial Livestock Services in West Timor demonstrated a seroprevalence to HPAI in the years 2004, 2005 and 2006 of 72.2% (95%CI: 66.2, 77.6), 13.2% (95%CI: 9.7, 17.4), and 18.0% (95%CI:10.1, 28.5) respectively. However, no evidence of the presence of disease has been reported since 2007. No evidence of disease or infection has also been reported in the neighbouring country, Republic of Democratic Timor Leste, since 2005 (Amaral, 2011). Disease freedom in Timor Leste is important because of the potential for cross-border movements of poultry between the two countries.

In response to the H5N1 outbreak in West Timor in 2004 to 2006 vaccination was not implemented, instead slaughter/stamping out was undertaken based on the Government's control strategic plan and regulation in 2004 (Decision of Directorate General of Livestock No:17/Kpts/PD.640/F/02.04 Guidelines of prevention, control and eradication of zoonotic disease of AI) (Directorate of

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Animal Health Indonesia, 2012). Since these initial cases no other clinical cases have been reported in the Province (unpublished data of the Provincial Livestock Services, NTT). Besides slaughter, the Government implemented movement controls of chickens and the importation of chickens was prohibited from infected areas, with importation of day old chickens (DOCs) only permitted from high biosecurity level, HPAI test-negative flocks approved and inspected by the Provincial Government. No day old ducks (DODs) are imported into West Timor.

According to the Terrestrial Animal Health Code, (OIE, 2015) a country, zone or compartment can be considered free from infection in poultry when: infection with HPAI viruses in poultry have not been present in the country, zone or compartment for the preceding 12 months, although the LPAI virus status may be unknown; or based on surveillance, any virus detected has not been identified as HPAI virus; or if infection has occurred in poultry in a previously free country, zone or compartment, HPAI free status can be regained three months after a stamping-out and disinfection policy has been applied, providing that surveillance has also been carried out during that period.

Analysis of the results of structured representative surveys can be used to demonstrate a zone or country is free from disease (Martin *et al.*, 2007). To support freedom from HPAI, a surveillance program targeting all susceptible species is required in a country/zone/region. This surveillance program can be carried out using appropriately designed randomised sampling techniques or by using targeted surveillance of high risk species in specific locations or birds/farms

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undertaking high-risk practices (OIE, 2010). In order to provide evidence that HPAI H5N1 was absent from West Timor, targeted surveillance was undertaken in high-risk areas comprising live bird markets (100 samples) and villages and commercial farms (200 samples). The results of this surveillance are reported and discussed in this study.

2. Materials and Methods

2.1. Study Area

West Timor is situated on the island of Timor, between Australia and the Democratic Republic of Timor Leste, and its economy is predominantly agricultural. Based on the animal census in 2013, poultry represented 90% of the total livestock population in West Timor (Central Bureau of Statistics of Nusa Tenggara Timur, 2013). Village/backyard reared chickens, commercial chickens (broilers and layers), and ducks are the most common types of poultry raised by households in West Timor. Commercial broiler chicken production is categorized as sector 3 (FAO, 2004) with low biosecurity and flocks of up to 5000 birds. Approximately half of these chickens are usually sent to a live bird market (LBM) for sale and the other half directly to restaurants. In West Timor most villagers keep small flocks of poultry under backyard settings (categorized as Sector 4) (FAO, 2004) and birds are used for home consumption or are also sold through the LBM to provide income (Provincial Government of Nusa Tenggara Timur (NTT), 2010). However, similar backyard production systems to those adopted in NTT have been reported to have low biosecurity and a high risk of infectious diseases (Conan *et al.*, 2012).

ACCEPTED MANUSCRIPT

This study was undertaken in two (City of Kupang - Kota Kupang; and Belu, which borders Timor Leste) of the five districts in West Timor and involved sampling village (backyard) and commercial poultry from five subdistricts, 10 villages and five markets (Figure 1). Alak, Maulafa and Kelapa Lima were the subdistricts selected from Kota Kupang, while the subdistricts of Tasifeto Barat and Kota Atambua were selected from the district of Belu. Four villages were selected from the district of Belu (Naitimu, Bakustulama, Tenukiik and Manumutin), while from Kota Kupang six villages were selected (Alak, Namosain, Sikumana, Naikolan, Lasiana and Oesapa) (Figure 1). The selection of the districts, subdistricts, villages and markets included in this study was made using a targeted sampling approach, where high-risk locations and live-bird wet markets were specifically targeted for sampling. These districts and locations were classified as high-risk based upon previous reports and through discussions and findings documented by the Provincial Government Livestock Services of NTT. Kota Kupang was considered to be a high-risk district due to the previous presence of HPAI and it also is the main poultry production region in NTT. Belu was selected due to the risk of cross-border movement of poultry with Timor Leste. Five LBM (one market in the district of Belu and four markets in the district of Kota Kupang) were also selected. The LBM selected were Baru Market in Belu, and Inpress Naikoten Market, Oebobo Market, Oeba Market and Oesapa Market in Kota Kupang. These LBM were selected because they were the largest in these districts and hence were considered to have the highest risk of having infected birds, if infection was present. At the LBM both village chickens and

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commercial broilers were sold. The selection of owners in the villages whose birds were sampled was based upon previous reports of disease in their birds and advice provided by the Provincial Government Livestock Services of NTT of a higher risk in this group. All birds were clinically healthy and no vaccination against AI had been applied in West Timor prior to the survey.

2.2. Study design

2.2.1. Sample Size Calculation

The total number of birds sampled per village and per market was 20. This sample size was based on the formula for freedom testing with adjustment for test sensitivity (84.3%) and specificity (97.7%), estimated village poultry population of 1000, desired village sensitivity of 95% and a level of confidence of 95%. The design prevalence was set at 20% (Sergeant, 2013).

2.2.2. Sample Type and Test Methods

Cloacal and tracheal swabs were taken from live birds and tested immediately with the pen-side Anigen® Rapid Test Kit as described by Meijer (2006). The Anigen Rapid® Avian Influenza Virus Antigen Test Kit is a chromatographic immunoassay for the qualitative detection of avian influenza type A virus antigen in avian cloacal or tracheal swabs (BioNote. Inc, Seoul, Republic of Korea). The test has a reported sensitivity of 100% at the farm-level (Meijer, 2006) and sensitivity of 84.3% (95% CI, 78.1-88.9%) and specificity of 97.7% (95% CI, 94.2–99.1%) at the individual bird level (Meseko *et al.*, 2010). In Indonesia,

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Anigen® has been the preferred test because it is easy to use, and has adequate sensitivity and specificity (Boland *et al.*, 2006).

2.3. Sampling, sample collection, and the test procedures

A total of 300 swabs were collected from poultry (292 chickens and 8 Muscovy ducks) from the 10 villages and 5 markets between August and October 2013. Ten "farms" with poultry from each village were selected based on a previous history of disease or advice from the Provincial Government Livestock Services of NTT that they represented a high risk group (total of 100 "farms"). Two birds were sampled on each of the selected farms. The birds were caught by the farmers (convenience sampling). Only 8 (2.67%) samples were collected from Muscovy ducks as few were present on the sampling days.

Two chickens were sampled from each of ten vendors randomly selected at each of the five LBM selected (total of 50 vendors, 100 chickens). These five LBM are the largest markets selling chickens in the locations. Samples from an individual village or market were all collected on the one day.

Sterile cotton swabs were initially inserted and rubbed on the mucosa of the trachea and the same swab was then inserted into the cloaca and gentle pressure applied while rotating the swab two or three times on the side of the cloaca. The procedure for testing was based on the recommendation of the manufacturer.

The level of HPAI freedom was calculated using FreeCalc in Survey Toolbox (Cameron, 1999).

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3. Results

All swab samples were negative on testing with the Anigen® Rapid test (0%; 95% CI: 0.0-1.2%). One sample in Naitimu gave an invalid result and therefore was retested. On retesting the result was negative.

The probability of detecting 0 positive samples in a sample of 300 from an infected population with a prevalence of 20% in a population of 10,000 was 0.0000 (95%CI: 0.0000-0.0122). Consequently it can be concluded with a high level of confidence (1.0, 95%CI: 0.9878, 1.0) that this population is not infected, and that at the time of sampling West Timor was highly likely to be free from HPAI.

4. Discussion

Targeted surveillance is defined as a sampling approach where samples (animals or sites) are specifically selected for testing (Williams *et al.*, 2009). This form of surveillance increases the probability of detecting infection, if present. In this study, the high-risk areas included markets and backyard and commercial poultry farms. Backyard farms (village chickens) have been shown to play an important role in the transmission of HPAI H5N1 in Thailand (Tiensin *et al.*, 2005) and LBM have also been recognized as significant places for the maintenance and exchange of H5N1 in Indonesia (Sims *et al.*, 2005). Markets in West Timor are wet markets where poultry (live commercially reared, village and backyard free-range chickens) and other animals, as well as other agricultural products and commercial products, are mixed together. Some of these poultry would return to

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their owner's home if they were not sold, and could potentially transmit virus from the markets back to the farms (Roche *et al.*, 2014).

Commercially available rapid antigen tests for influenza A are quick and simple to perform allowing the prompt diagnosis of HPAI for the early detection and containment of disease, factors that are critical in disease control (Meseko *et al.*, 2010). The high sensitivity of the Anigen® test makes it suitable for use in a field situation for testing or screening of flocks (Loth *et al.*, 2008) or groups of birds as in the current study. However, the test detects both HPAI and LPAI isolates, therefore negative results provide further confidence in the freedom of the sampled population from AI.

Freedom from a disease is usually defined as the true prevalence being below a specified design prevalence with a certain level of confidence (More *et al.*, 2009). A country can be considered free from a disease based on historical evidence (absence of the disease or infection for a certain period of time) (OIE, 2010) along with the results of surveys (Cannon, 2002). In the current study the minimum expected prevalence (MEP) was set at 20% to provide strong evidence that the disease under study was not present (Martin and Cameron, 2002) and to increase the chance of detecting disease if it was present through a larger sample size (Cannon, 2002). As the disease is known to affect over 50% of a population of chickens in an outbreak (Taubenberger and Morens, 2009), selecting a lower value strengthens even further the confidence that the poultry population sampled was free from HPAI. In the future more targeted sampling of ducks should be

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undertaken because of their role in the transmission of HPAI (Gilbert *et al.*, 2006; Kim *et al.*, 2009).

No cases of HPAI H5N1 have been reported in West Timor since 2006. Besides the use of historical data, designed targeted surveillance was used to support the hypothesis that West Timor was free from HPAI. As no positive results were found in this targeted surveillance of a high-risk population conducted in West Timor, there is a high level of confidence in the region being free from H5N1 infection at the time of sampling.

In West Timor, even though an outbreak of H5N1 occurred in 2004, surveillance of H5N1 was not implemented until 2005 (Personal communication Drh. Cahyo Sunarno, Coordinator of the Participatory Disease Surveillance and Response (PDSR) program of Nusa Tenggara Timur Province). This surveillance was performed through the PDSR program, which was designed to strengthen veterinary services and empower communities in order to prevent and control HPAI primarily targeting the sector 4 poultry industry (household level) (Azhar *et al.*, 2010). All samples collected as part of that surveillance have also been test negative; however no surveillance has been undertaken in wild birds and future surveillance activities should include samples from such birds as spill-over of H5N1 from migratory birds has been reported (Munster *et al.*, 2006; Gilbert *et al.*, 2010).

It is likely that the sampling of birds on farms was not representative of the total population as birds, with clinical or subclinical disease, would have been easier to

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catch, although in this study all sampled birds appeared clinically healthy. However this potential sampling bias would have increased the likelihood of detection of virus, if present.

It is concluded from this study that it is highly unlikely that HPAI H5N1 was present in West Timor at the time of the study. Active surveillance should be continued to ensure that West Timor maintains this apparent disease-free status, along with improved biosecurity on commercial and backyard farms and implementation of programs to increase the awareness of the disease by backyard poultry owners.

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ACCEPTED MANUSCRIPT

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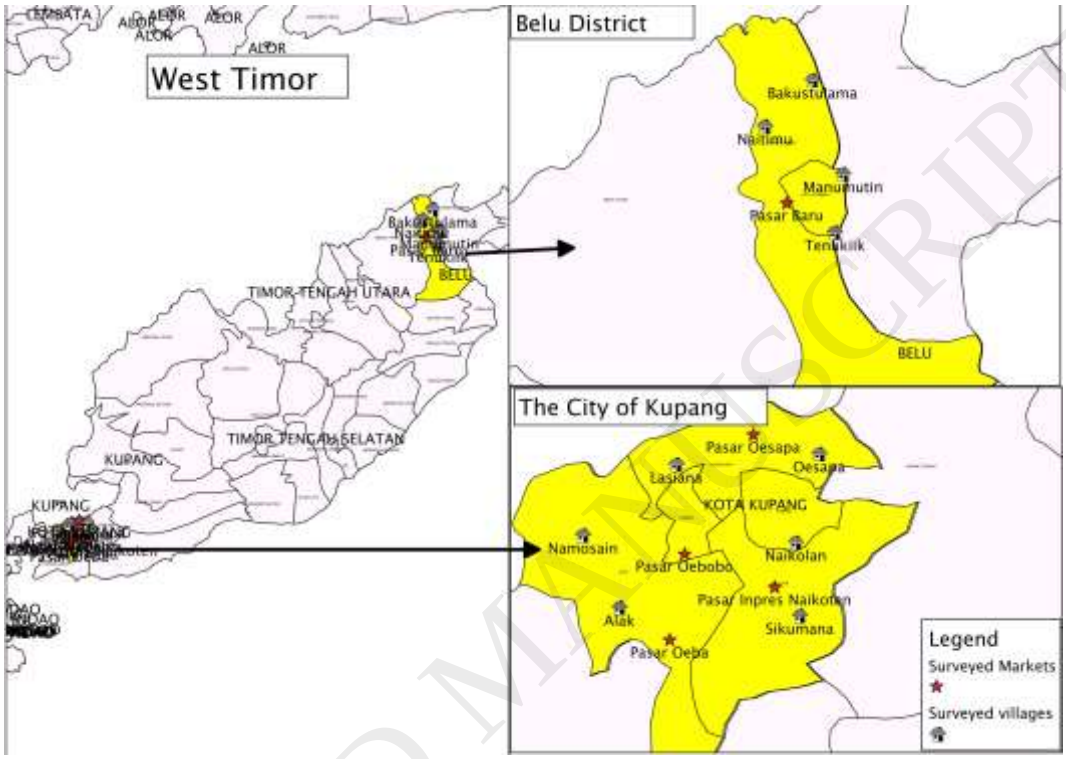
Caption of Illustration

Figure 1. Map of West Timor showing the location of villages and markets of Kota Kupang and Belu where sample collection was undertaken



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